Fungal Biology 123 (2019) 497–506

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Amblypygid-fungal interactions: The whip spider exoskeleton as a substrate for fungal growth

Alastair T. Gibbons [a], Alexander Idnurm [b], Michael Seiter [c], Paul S. Dyer [a], Matthew Kokolski [a], Sara L. Goodacre [a], Stanislav N. Gorb [d], Jonas O. Wolff [e, *]

[a] School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom
[b] School of BioSciences, University of Melbourne, Parkville Campus, Melbourne, VIC, 3010, Australia
[c] Department of Integrative Zoology, University of Vienna, Althanstraße 14, A-1090, Vienna, Austria
[d] Functional Morphology and Biomechanics, University of Kiel, Am Botanischen Garten 9, D-24118, Kiel, Germany
[e] Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia

ARTICLE INFO

Article history:
Received 15 February 2018
Received in revised form
11 April 2019
Accepted 1 May 2019
Available online 10 May 2019

Corresponding Editor: Gabor M. Kovacs

Keywords:
Arachnida
Arthropod-fungi relationship
Cladosporium
Mucoromycota
Mycoflora
Simplicillium

ABSTRACT

Fungi and arthropods represent some of the most diverse organisms on our planet, yet the ecological relationships between them remain largely unknown. In animals, fungal growth on body surfaces is often hazardous and is known to cause mortality. In contrast, here we report the presence of an apparently non-harmful mycobiome on the cuticle of whip spiders (Arachnida: Amblypygi). The associations are not species-specific and involve a diversity of fungal species, including cosmopolitan and local decomposers as well as entomopathogens. We discuss the ecology of the detected fungal species and hypothesize that the thick epicuticular secretion coat of whip spiders (the cerotegument) promotes fungal growth. It is possible that this relationship is beneficial towards the host if it leads to parasite control or chemical camouflage. Our findings, which are the first from this arthropod lineage, indicate that non-pathogenic interactions between arthropods and fungi may be much more widespread than predicted and call for more studies in this area.

1. Introduction

Fungi play an important role in ecosystems as decomposers and parasites and are one of the most diverse groups of microorganisms (Blackwell, 2011). They are ubiquitously present in the environment yet in many cases their relationships with other organisms are poorly understood, in particular how they interact with the most diverse and abundant group of animals, the arthropods. Entomopathogenic fungi often cause mortality (Boomsma et al., 2014; Evans, 2013; Samson et al., 2013; Vega et al., 2012) and have also been reported to manipulate the behaviour of the host. In the example of *Ophiocordyceps unilateralis*, infection has been observed to cause the death grip behaviour in carpenter ants (*Camponotus* spp.) (Hughes et al., 2011; Shang et al., 2015). Furthermore, digestion-assisting yeasts have been reported from insect guts (Suh et al., 2005), as well as the fungus gardens of ants and termites where plant material is processed and cultivated as a food source (Aanen et al., 2002; Batra and Batra, 1967; Fisher et al., 1996; Graham, 1967; Weber, 1966). A variety of fungal species have been reported as pathogens of insects, for example species in the genera *Metarhizium* and *Beauveria*, and some used for biocontrol purposes (Castrillo et al., 2011; de Faria and Wraight, 2007; Ferron, 1978; Lacey et al., 1996). By contrast, much less is known about potential pathogenic relationships with the Arachnida (Evans, 2013). Nevertheless, studies in this area have successfully established the use of *Beauvaria bassiana*, *Hirsutella thompsonii* and *Neozygites* spp. as both Acari-specific and non-specific pathogens (Chandler et al., 2000; Eken and Hayat, 2009; Gerson et al., 2008; Irigaray et al., 2003). Furthermore, there are reports of fungal pathogens of spiders (Bibbs et al., 2013; Evans, 2013; Greenstone et al., 1987; Nentwig, 1985; Samson et al., 2013) as well as anecdotal reports indicating that spiders and harvestmen are vectors for pathogenic fungi (Vanderwolf et al., 2016; Yoder et al., 2009).

The overwhelming majority of arachnid fungal pathogens are classified within the Pezizomycotina. They generally infect the host

* Corresponding author.
E-mail address: jonas.wolff@mq.edu.au (J.O. Wolff).

https://doi.org/10.1016/j.funbio.2019.05.003
1878-6146/

by adhering to the cuticle, penetrating it via a combination of enzymatic degradation and mechanical pressure, and then finally produce conidia on the surface of the cadaver (Askary et al., 1999; Evans, 2013). Evans (2013) suggested that fungal pathogens of arachnids are likely to be highly host specific and that specificity operates at the level of the exoskeleton. Observations of arachnid fungal pathogens indicate that the opisthosoma (the abdomen) is particularly vulnerable to fungal colonisation and this is possibly due to differences in cuticle structure compared to other parts of the host. A study of the cuticle composition of different body regions of the spider *Cupennius salei* reported that the prosoma, metatarsus and femur tissue had a varying degree of hardened exocuticle (Barth, 1973). In contrast, the epi- and exocuticle were absent in the opisthosoma, and instead was found to be predominantly composed of a mesocuticle. This layer is a non-hardened structure that is not highly sclerotised and lacks the waxy patches found on the procuticle or epicuticle (Capinera, 2008; Foelix, 1979). As adult insects generally have a highly sclerotised and tough exocuticle, Evans (2013) speculated that such differences in the composition of the cuticle might be sufficient to account for the presence of different arachnid and insect fungal pathogens.

An arachnid group that attracts attention is the order Amplypygi, commonly referred to as whip spiders. These are small to large arthropods that act as secondary and tertiary consumers in tropical and subtropical ecosystems, with over 200 species recognised (Bloch and Weiss, 2002; Chapin, 2014; Chapin and Hebets, 2016; Harms, 2018; Porto and Peixoto, 2013). In contrast to the majority of arachnids, whip spiders have a dorso-ventrally flattened body with highly sclerotised plates (tergites) along their abdomen and secrete a thick epicuticular cement layer (cerotegument) that contains complex micro- and nanostructures (Wolff et al., 2016, 2017).

With the increasing number of studies being undertaken on species in the order Amplypygi, certain interactions between whip spiders and other taxa, namely parasites and parasitoids, have become apparent. For example, infestation by a mite of the genus *Odontacarus* and egg laying by a parasitoid chloropid fly of the genus *Pseudogaurax* lead to mortality of whip spider hosts (Gonçalves-Souza et al., 2014; Rayor and Taylor, 2006; Viquez and de Armas, 2009). However, there have so far been no reports of bacterial or fungal relationships, either pathogenic or beneficial, with whip spiders (Chapin and Hebets, 2016).

During a comparative study of the cerotegument structure of whip spiders (Wolff et al., 2017), an unusually high level of fungal hyphae was observed growing on the exoskeleton. This is usually not found on the cuticle of other arachnids or insects (e.g. Wolff and Gorb, 2016). While a white substance covering the cuticle of individuals of the cave-dwelling whip spider *Phrynus longipes* had also previously been observed by Chapin and Hebets (2016), leading them to remark on the potential for fungal-amblypygid relationships, these observations were not discussed further presumably because the finding was interpreted as an unusual event. Here we studied the fungal community for the first time on the cuticle of a variety of whip spiders that originate from several geographic regions, namely Southeast Asia, Central America, Africa and Australia.

2. Material and methods

2.1. Scanning electron microscopy

Scanning electron microscopical images, acquired from previous surveys of cerotegument and cuticular structures of various amblypygid species (Wolff et al., 2015a, 2017), were analysed for the presence of fungal hyphae and spores. The material comprised of the exuviae from 12 species, ethanol-preserved material of two species from two field collections and living animals of three species from the breeding stock of M. Seiter. Samples for microscopy were prepared from air-dried, critical-point dried or cryo-fixated material, and were studied with a Hitachi S-4800 scanning electron microscope (Hitachi Ltd., Tokyo, Japan) in warm or cryo-mode. For a list of materials see Table 1; for further details on animal origin, housing and sample preparation, see Wolff et al. (2015a, 2017). The cuticle of an individual of *Charinus pescotti*, wild caught in Kuranda, Northern Queensland (the same population as animals used for fungal isolation, see below), was inspected, being directly fixed in 70 % ethanol during the collection in the field. Pieces of the carapace and legs were critical point dried, sputter coated with chromium in a Q 150T ES (Quorum Technologies Ltd, Laughton, UK) and visualized in a JEOL JSM- 7100F field emission scanning electron microscope (JEOL USA, Peabody, MA, USA).

To determine the location of fungal hyphae on and in the whip spider body, scanning electron microscopy of a sectioned piece of leg was performed. For this purpose, a living individual of *Charinus cubensis* was anaesthetized with carbon dioxide and legs were clipped off using micro scissors. Legs were immediately fixed with buffered 2.5 % glutaraldehyde and 1 % osmium tetroxide, dehydrated in a series of increasing ethanol concentrations and embedded in Epon. Epon blocks were trimmed with a Leica EM TRIM2, and 1.5 μm sections were made using a Leica EM UC7 ultramicrotome with a Diatome MC2391 diamond knife (Diatome AG, Biel, Switzerland) to about a half of the embedded specimen. The remaining specimen blocks were bathed for 1.5 h in a concentrated KOH solution in two parts of methanol and one part of propylene oxide to remove the epoxy resin (Maxwell, 1978), then put in absolute ethanol and critical point dried. Samples were sputter coated with 10 nm Au-Pd in an EM SCD 500 (Leica Microsystems, Mannheim, Germany) and viewed in a Hitachi S-4800 scanning electron microscope.

2.2. Isolation of fungal strains

Fungi were isolated from living amblypygids and exuviae and then cultivated on agar plates. Whip spiders were obtained from the following sources: the breeding stock of M. Seiter (Vienna, Austria) *Damon annulatipes*, *Acanthophrynus coronatus*, *Phrynus exsul*; a UK-based breeder of whip spiders (J and G. Smith, Metamorphosis, High Wycombe) *Damon diadema*; and wild caught by an Australian collector (A. Henderson, Minibeasts, Kuranda) *C. pescotti*. The following methods were used to isolate fungi from whip spiders: (1) pushing the dorsal side of an individual onto the medium, with and without previous cleaning (cleaning was performed by blowing the surface of the host with pressurized sterile air and/or wiping with a 70 % ethanol swab), (2) allowing an individual to walk, encouraging tarsal contact, on the agar plate for 10 s, (3) whilst held, gently shaking an individual to dislodge any loose spores, (4) swabbing an individual with a cotton stab wet with sterilized water + 0.1 % Tween 20, (5) scraping the dorsal surface of an individual with a scalpel, (6) placing pieces of cuticle onto the medium. Furthermore, fungi were isolated from the material the whip spiders were kept or transported on (bark, tissue, moss) to test if the fungal species found on individuals were present in their environment.

Fungi were cultured on potato dextrose agar with added ampicillin (100 μg/ml) to inhibit bacterial growth. In the case of *C. pescotti*, isolates were cultured on five types of agar media; 1 % yeast extract + 2 % peptone + 2 % dextrose YPD, 10 % Campbell's V8 juice, Difco potato dextrose, Difco yeast nitrogen base with chitin (0.5 %) as the carbon source, and water agar. The proportions used for the Difco media were as according to the manufacturer's

Table 1
Summary of the observation of putative fungal structures by scanning electron microscopy on the cerotegument of whip spiders.

Amblypygi species	Origin	Type of sample	Observed fungal structures (presence indicated by a cross)					
			Septate hyphae		Hyphae without constrictions	Conidiophores	Spores / Conidia	
			Cells cylindrical	Cells rounded			Smooth	Micro-ornamented
Acanthophrynus coronatus	Bred in Austria	Dry exuvia			X			
Charinus acosta	Bred in Austria	Dry exuvia		X		X	X	X
C. cubensis	Wild caught in Cuba, kept in Austria and Germany	Fixed alive, sectioned			X		X	
C. neocaledonicus	Collected in New Caledonia	Ethanol preserved		X	X	X	X	
C. pescotti	Wild caught in Australia	Ethanol preserved	X	X	X	X	X	X
Charon cf. *grayi*	Bred in Austria	Dry exuvia	X	X	X	X	X	X
	Kept in Austria and Germany	Cryo-fixation of living animal		X		X	X	X
	Kept in Austria	Ethanol preserved	X	X			X	X
Charon cf. *grayi* prenymph	Kept in Austria	Ethanol preserved					X	
Damon annulatipes	Bred in Austria	Dry exuvia	X	X	X	X	X	X
Heterophrynus sp.	Bred in Austria	Dry exuvia	X	X		X	X	X
Phrynichus dhofarensis	Wild caught in Oman, kept in Austria	Dry exuvia		X				
P. aff. *barbadensis*	Bred in Austria	Dry exuvia		X		X	X	X
P. damonidaensis	Wild caught in Cuba, kept in Austria	Dry exuvia	X					
P. decoratus	Wild caught in Cuba, kept in Austria	Dry exuvia	X					
P. exsul	Bred in Austria	Dry exuvia		X		X	X	X
P. longipes	Bred in Austria, kept in Germany	Cryo-fixation of living animal		X				
	Kept in Austria	Dry exuvia		X				
P. marginemaculatus prenymph	Bred in Austria	Ethanol preserved					X	
Sarax curioi	Wild caught in Philippines, kept in Austria	Dry exuvia		X				X
Sarax brachydactylus	Wild caught in Philippines, kept in Austria and Germany	Cryo-fixation of living animal		X				

instructions (39 g/L PDA and 6.7 g/L YNB), and these media were supplemented with rifampicin (50 μg/ml). This selection of media was chosen to favour the cultivation of a diversity of fungal taxa. Cultures were grown at 18 °C for three to seven days before isolates with different morphological appearances were sub-cultured. For sporulating or yeast forms, strains were sub-cultured from single cells isolated by streaking onto new media. For filamentous forms, hyphal tip sub-culturing was used for strain isolation.

2.3. Identification of fungal species

To examine the strains microscopically a portion at the edge of a sporulating colony, near the periphery of the matured spores, was cut out using a dissecting needle and platinum wire just below the agar surface. This was placed onto a slide with a 70 % ethanol solution. Once the ethanol had evaporated, a drop of lactic acid was placed onto the sample and a coverslip was used for viewing under a light microscope (Motic BA310E) at 40× magnification and using phase contrast. Photos were taken using a Moticam 3.0MP digital camera.

Fungal isolates were cultured in YPD broth or *Aspergillus* complete medium (ACM) (Paoletti et al., 2005) and genomic DNA was extracted as previously described (Murtagh et al., 1999; Pitkin et al., 1996). The internal transcribed spacer (ITS) region of nrDNA, used routinely for fungal identification (Schoch et al., 2012), was amplified with the primers ITS1 (Gardes and Bruns, 1993) and ITS4 (White et al., 1990). The PCR products were sequenced using Sanger chemistry at the DNA Sequencing Facility, Queen's Medical Centre, University of Nottingham and chromatograms were inspected and trimmed using MacVector 14. The sequences obtained were compared to the GenBank nr database and UNITE version 7.2 database (Kõljalg et al., 2013) using BLASTN. If strains could not be resolved by the analysis of ITS sequence alone, then further loci were amplified and sequenced. These included regions of the β-tubulin and actin genes, amplified with primer pairs β-tubulin BT2a-F, β-tubulin BT2b-R (Glass and Donaldson, 1995) and actin ACT-512F, actin ACT-783R (Carbone and Kohn, 1999). Based on the consensus regions present in translation elongation factor 1-α sequences (*tef1*) of a variety of *Cladosporium* species in Bensch et al. (2012) a *Cladosporium*-specific primer pair was designed; Clad EF1-alpha F CACCCCGCCTCGTCGCAA, Clad EF1-alpha R short GGAGTCTCGAACTTCCAGAG. Obtained sequences were deposited into GenBank (see Suppl. Table 1 for GenBank accession numbers). A representative set of strains were sub-cultured by swabbing spores from pure cultures and streaking them onto fresh media before being deposited at CABI, Kew Gardens, UK (accessions IMI 506802 – IMI 506806) or the Queensland Plant Pathology Herbarium (BRIP), Australia (accessions BRIP 66197 – BRIP 66208). Three *Cladosporium* isolates were not deposited due to the samples failing to culture and isolates belonging to the genera *Penicillium* were not deposited due to being frequently isolated from the environment as well as the whip spider host. In addition, for strains that were genetically similar, only a single strain was deposited for each species.

3. Results

3.1. Scanning electron microscopy reveals extensive fungal colonisation on whip spiders

Fungal growth on whip spiders has not usually been distinguishable by the naked eye, but in rare cases was growth so

excessive that it was visually obvious (Fig. 1A). However, by scanning electron microscopy characteristic fungal structures were commonly observed (Table 1; Fig. 1B–I). Inspection of sections and fractures of animal pieces revealed that fungal structures were only present in and on the cerotegument layer, but never invaded the cuticle or body (Fig. 1I). Notably, none of the whip spiders or their exuviae were free of putative fungal hyphae. This applied equally to fresh and preserved material or wild-caught and captive-bred animals. In most samples both hyphae and putative conidiophores, as well as spores, were found, indicating actual fungal growth on the cuticle. The frequent and simultaneous occurrence of hyphae with different morphologies indicated the likely co-occurrence of different fungal species. The presence of hyphae without regular constrictions (i.e. lack of septation) was a possible indication of the occurrence of coenocytic hyphae.

3.2. Isolation and identification of fungal strains

Fungal strains were isolated from whip spiders that had been bred in captivity in two European countries and collected from the wild in Queensland, Australia (Fig. 2). Individual animals yielded a diverse set of strains, illustrated by the variation in colony growth rates, morphology and appearance in cultures (Fig. 2A–L). With one exception of a yeast species, which was exclusively cultured on the chitin-based medium, these showed filamentous growth and most produced asexual spore structures (Fig. 2M–P).

Taxonomic analysis based on ITS, β-tubulin, EF-1a and/or actin sequences amplified from the samples led to the identification of at least 25 different putative fungal taxa from whip spiders, their exuviae or housing/transport materials (Table 2). Most sequences showed between 95 and 100 % identity to known species.

The majority of the isolates were in the Pezizomycotina, representing the genera *Acremonium*, *Aspergillus*, *Cladosporium*, *Lecanicillium*, *Metarhizium*, *Neopestalotiopsis*, *Penicillium*, *Simplicillium* and *Trichoderma*. In addition, several species in the Mucoromycota representing the genera *Mortierella*, *Mucor* and *Syncephalastrum*, and a single isolate from the Saccharomycotina in the genus *Candida*, were also found. Putative species from the genus *Cladosporium* were isolated from bred, wild caught and the shed exuvia of whip spiders (Table 2). *Lecanicillium fungicola* was isolated from the exuvia of *D. diadema* but was not found on captive bred whip spiders. Two species of *Simplicillium* were found on wild-caught *C. pescotti*. Species from the genera *Mucor*, such as *Mucor racemosus* and *Mucor fragilis*, were isolated from both bred and wild caught whip spiders.

In general, animals from breeding stocks showed a lower number of different fungal taxa than the wild caught *C. pescotti*. The technique of scraping wild caught individuals yielded a higher number of fungal taxa, per individual, in comparison to swabbing (Table 2). From the captive bred individuals, regardless of treatment, often only a single fungal taxon was cultured. Analyses of housing or transport materials the individuals were kept in

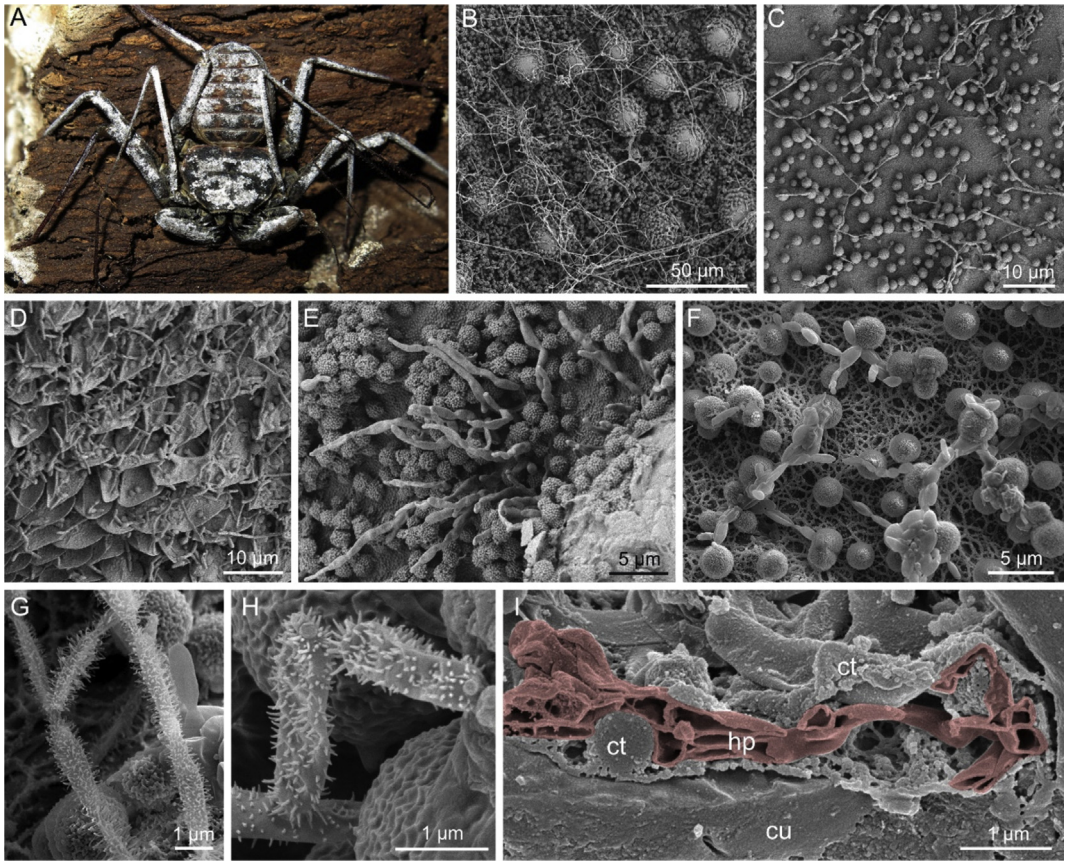

Fig. 1. Fungal growth on whip spiders (Amblypygi). (A) Photograph of a living *Phrynus barbadensis* (Phrynidae) from our breeding stock showing extreme fungal growth. (B–I) Scanning electron micrographs of characteristic fungal structures on cuticular surfaces in whip spiders. Globular structures are part of the whip spiders cement coat (cerotegument). (B) *Phrynus decoratus*, exuvia of carapace. (C) *Sarax brachydactylus*, cryo-fixated leg. (D) *Charon* cf. *grayi*, cryo-fixated leg. (E) *Phrynichus jayakari*, exuvia of carapace. (F,G) *Charinus pescotti*, ethanol-fixed, carapace. (H) Micro-ornamented conidia on exuvia (leg) of a *Phrynus exsul*. (I) Detail of a section of a leg of a *Charinus cubensis* fixed in glutaraldehyde, showing the growths of hyphae (indicated by red hue for clarity) within the cerotegument coat. Abbreviations: ct, cerotegument; cu, cuticle; hp, hyphae. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

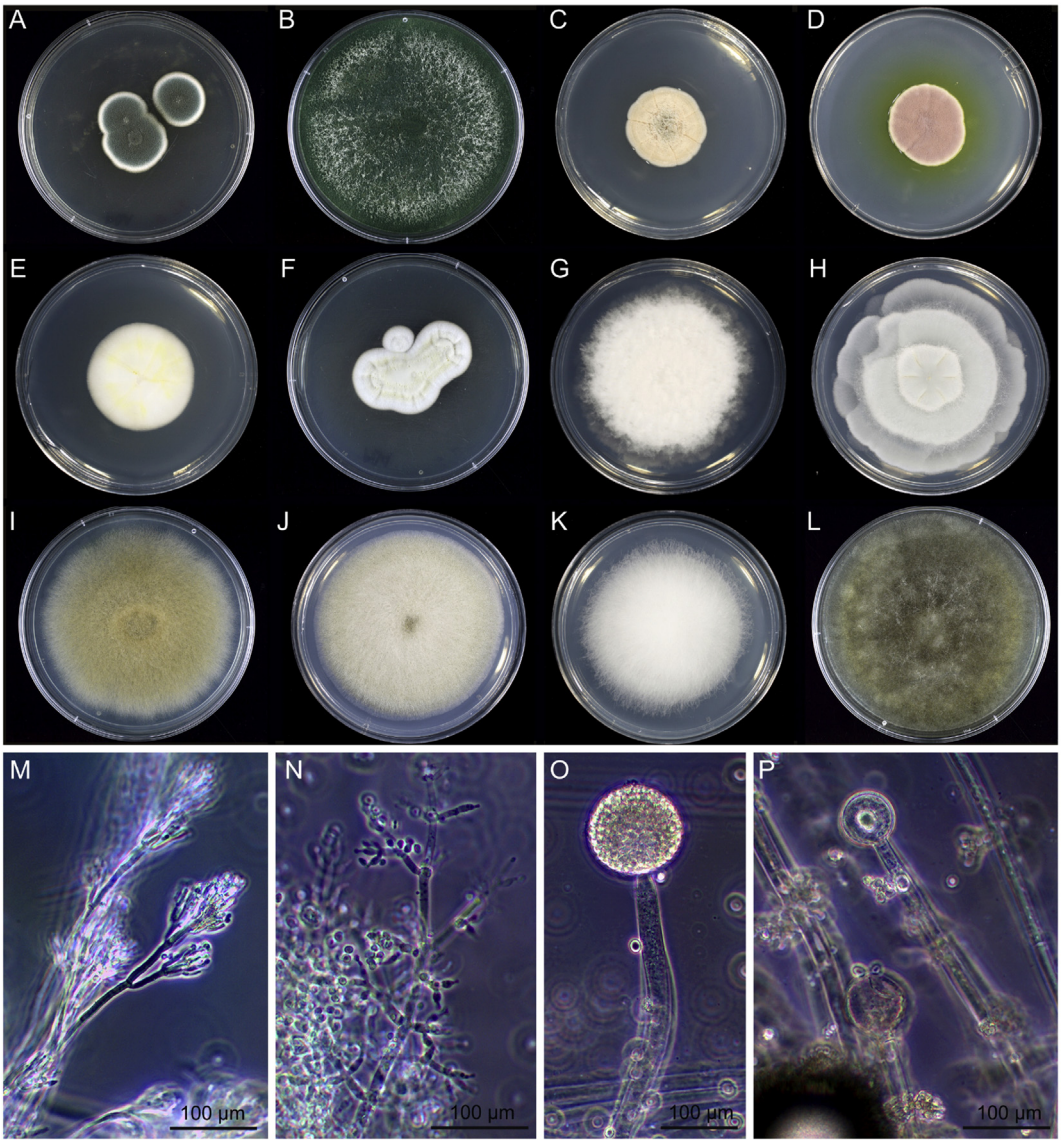

Fig. 2. Fungal isolates from whip spiders. (A–L) Diversity in growth patterns of fungi isolated from whip spider cuticle. The closest matches of the fungal ITS sequences in GenBank are provided. (A) *Penicillium* sp. from *D. diadema*. (B) *Trichoderma asperellum* from *D. diadema*. (C) *Acremonium persicinum* from *C. pescotti*. (E) *Simplicillium* sp. from *C. pescotti*. (D) *Metarhizium marquandii* from *C. pescotti*. (F) *Lecanicillium fungicola* from *D. diadema*. (G) *Neopestalotiopsis surinamensis* from *C. pescotti*. (H) *Mortierella exigua* from *C. pescotti*. (I) *Mucor racemosus* from *D. diadema*. (J) *Mucor fragilis* from *C. pescotti*. (K) *Mucor* sp. from *C. pescotti*. (L) *Syncephalastrum* sp. from *D. diadema*. (M–P) Microscopic images of fruiting bodies. (M) *Penicillium* sp. from *D. diadema*. (N) *Trichoderma* sp. from *D. diadema*. (O) *Mucor racemosus* from *D. diadema*. (P) *Syncephalastrum* sp. from *D. diadema*.

revealed the presence of similar, but less, fungal taxa than on the whip spiders themselves. For example, species from the genus *Mucor* were obtained both from wild caught *C. pescotti* and the sphagnum moss on which an individual was stored. Similarly, *Penicillium* spp., which are ubiquitous in the environment (Frisvad and Samson, 2004), were frequently isolated from captive-bred *D. diadema* and the bark from the terrarium (Table 2).

4. Discussion

Fungal hyphae and spores on the cuticle of whip spiders is not an anomaly but a frequent occurrence (Fig. 1). Fungi were consistently observed on whip spiders, regardless if they were wild caught or bred in captivity or originated from multiple continents. When cuticle samples from a variety of species were observed by scanning electron microscopy, characteristic fungal structures were present on all of the samples. We isolated 25 fungal strains from the cuticle of five whip spider species.

Based on the molecular taxonomic analyses, some of the samples matched genera known to contain arachnopathogenic species, including *Lecanicillium*, *Cladosporium* and *Mucor* (Bibbs et al., 2013; Eken and Hayat, 2009; Fernandes and Bittencourt, 2008). A key question concerning these and the other fungi isolated from the whip spiders was their origin. As outlined below, no strain was isolated that could be considered unique in its association with whip spiders. Instead, there was a general trend that many of these fungi can be commonly isolated from environmental sources, whilst noting that some have previously been shown to have associations with arthropods (Evans, 2013; Koike et al., 2004; Vega et al., 2008). It is acknowledged that our approach is limited to fungi that can be cultivated *in vitro* and the actual mycobiome may be more diverse. Also we cannot exclude contamination of the cuticles by environmental sources (although attempts were made

Table 2
Origins of fungal strains isolated from whip spiders and putative identifications based on BLAST matches (GenBank and UNITE databases) of DNA sequences. See Supplemental Table 1 for additional information.

Amblypygi species and origin	Animal treatment	Putative fungal species identification(s)[a]
Damon diadema:	Pushed on agar, no cleaning	*Penicillium* sp.
Bred in the UK, transported		*Mucor racemosus*
and housed with shredded bark		*Trichoderma asperellum; Trichoderma* sp.; *Syncephalastrum* sp.
	Animal shaken to dislodge any loose spores	*Cladosporium psychrotolerans*
	Walked on agar	*Penicillium* sp.
		Mucor racemosus
		Syncephalastrum sp.; *Aspergillus flavus; Aspergillus* sp.
	Cleaned with air, than pushed on agar	*Cladosporium ramotenellum*
	Cleaned with alcohol swab and air, then pushed on agar	*Penicillium* sp.
	Swabbed	*Trichoderma asperellum*
	Exuvia on agar	*Lecanicillium fungicola*
		Penicillium sp.
	Bark from terrarium	*Penicillium* sp.
Charinus pescotti:	Transported sterile - Swabbed	*Acremonium persicinum*
Wild caught in Australia	Transported sterile - Scraped	*Mortierella* sp.
		Neopestalotiopsis surinamensis
		Cladosporium aciculare
		Simplicillium sp.
		Metarhizium marquandii
	Transported sterile with wet tissue - Scraped	*Mortierella exigua*
	Transported sterile with wet tissue - Swabbed paper	*Simplicillium* sp.
	Transported with sphagnum moss - Swabbed	*Candida natalensis*
		Mucor fragilis
	Transported with sphagnum moss - Swabbed moss	*Mucor* sp.
Damon annulatipes:	Cuticle placed on agar	*Cladosporium* sp.
Exuvia from animal bred in Austria		
Acanthophrynus coronatus:	Cuticle placed on agar	*Penicillium* sp.
Exuvia from animal bred in Austria		
Phrynus exsul: Exuvia from animal bred in Austria	Cuticle placed on agar	*Penicillium chrysogenum*

[a] Where individual species could not be discriminated (i.e. multiple species showed 100 % identity) then only the genus name is listed e.g. *Penicillium* spp. Where only the genus name is listed, note that this does not imply that similar species were found on different samples.

to remove superficial fungi). Despite these caveats, a number of points about these interactions are worth raising.

An isolate of *L. fungicola* was obtained from the exuviae of the captive-bred whip spider *D. diadema* from the UK. Species within the genus *Lecanicillium* are pathogens of insects, nematodes, mushrooms and plants and thus have been commercially developed for the suppression of pest arthropod populations (de Faria and Wraight, 2007; Goettel et al., 2008). In arachnids, *Lecanicillium lecanii* has been trialled as a potential bioacarcide against the two-spotted spider mite *Tetranychus urticae* and can reduce their population under greenhouse conditions (Chandler et al., 2005; Shin et al., 2017). In addition, there has been a report of *L. lecanii* being used to control the cattle tick *Rhipicephalus microplus* with a potential for the control of engorged females, eggs and larvae of *R. microplus* (Angelo et al., 2010).

It has been demonstrated *in vitro* that some *Lecanicillium* species are able to produce toxic metabolites that are hypothesised to aid the fungus in overcoming its host (Claydon and Grove, 1982; Gindin et al., 1994). *L. fungicola*, which was isolated in this study, causes serious economic losses in commercially cultivated mushrooms, especially the white-button mushroom *Agaricus bisporus* (Berendsen et al., 2010). Although *L. fungicola* is phylogenetically closely related to a variety of insect pathogens, experimental evidence for the pathogenicity of *L. fungicola* is lacking. In addition, two isolates from the closely related genus *Simplicillium* were detected in the present study on wild-caught *C. pescotti*. The closest BLAST matches were to unpublished sequences in GenBank, derived from fungi of arthropod origin (see Suppl. Table 1).

In this study we isolated three different *Cladosporium*. *Cladosporium* species have recently been the subject of thorough phylogenetic analysis, and the genus currently has 218 recognised species split into three major species complexes (Bensch et al., 2015, 2018; Flannigan et al., 2016). These species have been reported from a range of substrates including human and animal clinical samples, soils, fruit, industrial water and indoor environments, and *Cladosporium* is considered among the commonest genera found in indoor and outdoor air (Bensch et al., 2018; Ma et al., 2017; Sandoval-Denis et al., 2016; Visagie et al., 2014). In addition some *Cladosporium* species have been identified as entomopathogens, being causal agents of disease of pest species such as whiteflies *Bemisia* spp., multiple aphid species, the coffee berry borer *Hypothenemus hampei* and there is preliminary evidence for pathogenicity against the two spotted spider mite *T. urticae* (Abdel-Baky, 2000; Eken and Hayat, 2009; Vega et al., 2008). Here, *Cladosporium psychotolerans* and *Cladosporium ramotenellum* were isolated from captive bred *D. diadema*. These *Cladosporium* species have been repeatedly isolated from indoor and outdoor environments and both also have a wide geographic distribution, currently being reported from three continents; Australasia, Europe and North America, and the latter also being known from Asia (Bensch et al., 2018). *Cladosporium aciculare*, which was isolated from the wild caught *C. pescotti*, is described as being similar to *Cladosporium coloradense*, a member of the *Cladosporium sphaerospermum* species complex, and interestingly has only been previously reported from *Syzygium corynanthum*, a tree species found only in Australia (Bensch et al., 2015, 2018).

Isolates in the genus *Trichoderma* were obtained from the captive-bred *D. diadema* and *Metarhizium* species from the wild-caught *C. pescotti*. Species within the genera *Trichoderma* and *Metarhizium* are well known entomopathogens and have been

developed as biological control agents against insects (Jackson et al., 2010; Ownley et al., 2010; Shakeri and Foster, 2007). *Trichoderma* spp. are common in soil and root ecosystems but have been found to be opportunistic plant symbionts and pathogens of other fungi (Harman et al., 2004). *Trichoderma harzianum* is a commercial biological control used to target insects and nematodes (Jassim et al., 1990; Santamarina et al., 2002; Suarez et al., 2004). *Trichoderma asperellum* was detected in the present study on the captive bred *D. diadema*. *T. asperellum* is one of the less studied species within the genus but is used as a microbial pesticide of seed-borne and stem rot diseases (Tsai et al., 2008; Yoshioka et al., 2012). More recently it has been reported to be successful in the management of root-knot nematodes for various crops (Affokpon et al., 2011) but evidence for it being an entomopathogen is lacking.

Similarly, *Metarhizium* species are well-documented entomopathogens and arachnopathogens with a widespread distribution (Meyling and Eilenberg, 2007; Roberts and St Leger, 2004). They are most commonly isolated from soil environments and have been extensively used as biological control agents in temperate regions, especially in agriculture and forestry ecosystems (Hajek, 1997; Lacey et al., 2001). *Metarhizium anisopliae* is a recognised pathogen of over 200 insect species (Roberts and Hajek, 1992) and produces a family of cyclic peptide toxins and destruxins (Kershaw et al., 1999). A high dose of artificial destruxin injection is lethal to the host (Roberts, 1966). Kershaw et al. (1999) found that *Metarhizium* spp. were pathogenic to tobacco hornworm, the desert locust and the vine weevil and mortality was determined by destruxin titre. *Metarhizium marquandii* was detected in the present study from the wild-caught *C. pescotti*. Although *M. marquandii* is most commonly isolated from the soil (Luangsa-ard et al., 2017) it has also previously been reported as a pathogen of a variety of invertebrates including the Asian citrus psyllid *Diaphorina citri* and Yellow fever mosquito *Aedes aegypti* (Gallou et al., 2016; Leles et al., 2010).

Two *Aspergillus* species (*Aspergillus flavus* and *A. carneus*) were isolated in the current study from the captive-bred *D. diadema*. *Aspergillus* is one of the most studied fungal genera, having a worldwide distribution and being common in soil, indoor environments and food products (Chen et al., 2017). Recent studies have suggested that *Aspergillus* species may be as effective as other well-recognised entomopathogenic fungi (Bawin et al., 2016). *A. flavus* was of particular interest because there have been independent reports of its entomopathogenic activity (Mensah and Young, 2017; St Leger et al., 1993; Yang et al., 2015). Notably, *Aspergillus* spp. also produce multiple extracellular chitinase enzymes that may degrade and allow penetration of the host (Kramer and Muthukrishnan, 1997; St Leger et al., 1993).

Finally various putative Mucoromycota species were obtained from both the captive-bred *D. diadema* and wild-caught *C. pescotti*. Strains included those within the Mucoromycotina and Mortierellomycotina subphyla (Spatafora et al., 2016). Of particular interest was the isolation of strains with 99 % ITS similarity to isolates of putative *M. fragilis* responsible for death of brown widow spiders (Bibbs et al., 2013). Strains representing another Mucoromycotina genus, *Syncephalastrum*, were also isolated. This is a small genus, the most common species, *Syncephalastrum racemosum*, having diverse ecological roles, including infection of human toe nails and as a biocontrol agent against nematodes (Baby et al., 2015; Huang et al., 2014).

Regardless of the previously described pathogenic associations, we never observed any evidence in the present study that fungi could cause lethal infection in whip spiders. This was consistent with observations of over 10 y of breeding whip spiders and collecting them from field trips around the world (M. Seiter, pers observations). Even the individual with heavy fungal growth illustrated in Fig. 1 showed no detrimental impact on survival, with the external fungi being lost after moulting. This contrasts with observations of spiders (Aranaea), where the visual observation of such excessive fungal growth is invariably correlated with death of the host (Nentwig, 1985). The fact that fungal hyphae were not found to penetrate the bodies of the whip spiders, but grew within and on top of the cerotegument layer, also rejects the hypothesis of a parasitic relationship. Instead, we suggest that the various fungal species identified in the present study (i.e. the whip spider mycobiome) may have resulted from environmental selection for certain fungal taxa able to survive and grow on the particular, potentially demanding, conditions of the whip spider body surface. For example, some of the *Cladosporium* species identified have previously been reported from osmotically-stressful environments (Bensch et al., 2012, 2015). Indeed, it is possible that the fungi observed might acquire nutrients from the cerotegument of the whip spider. This superficial cement layer is built of epicuticular secretions that form complex microstructures, which result in a super-hydrophobic and self-cleaning state of the cuticle (Wolff et al., 2016, 2017). Therefore, the whip spider body is usually free of detritus and dirt particles, and the only external contaminants (besides fungi) we observed were rare occurrences of pollen and bacteria. After moulting, the cerotegument is secreted within 1–2 d, and hyphae are only observable on the cuticle after this period (Wolff et al., 2016), supporting the possibility that fungal growth is aided by the epicuticular secretions. In the first instar of whip spiders, the prenymph, the cerotegument is not present, and we could not detect fungal hyphae (although putative spores were present) (Wolff et al., 2015b), which may further support this hypothesis that the whip spiders could promote fungal growth by their secretion(s). However, to date little is known about the chemical composition of the cerotegument of whip spiders.

It is also conceivable that the fungal mycobiome might play a role in the control of parasitic micro-organisms or avoidance of predators. Many of the fungi we isolated from whip spiders, such as those in the genera *Aspergillus*, *Cladosporium* and *Trichoderma* are related to those that produce secondary metabolites that inhibit other microbes (Keller, 2019).

Interestingly, Chapin and Hebets (2016) observed that Amblypygids have been found co-existing with several other species including birds, mammals, scorpions, ants and termites, being found in the nests and/or burrows of these species. With the exception of termites, whip spiders may be preyed upon by such species. However, in most instances they seem to be able to avoid predation (Chapin and Hebets, 2016). For example, *Charinus quinteroi* and *Charinus platnicki* have both been found in ant nests (Weygoldt, 2000). Of particular interest is the species *Phrynus gervaisii*, which has been found to be present in nearly half of the nests of *Paraponera clavata*, a particularly toxic bullet ant (LeClerc et al., 1987). In parallel, Ramírez (2014) noted the occurrence of fungal hyphae on the cuticle of some crab spiders (Thomisidae) that cover themselves with a crust of detritus, and hypothesised that this could provide a camouflage effect ('*fungal crypsis*'). It is conceivable that the mycobiome of whip spiders, which primarily inhabit dark places such as caves or otherwise are nocturnal (Chapin and Hebets, 2016), may have a similar function. Entomopathogenic fungi present on the cuticle of whip spiders could also provide an effective control against parasitoid insects, which are reported to occasionally attack whip spiders (Chapin and Hebets, 2016).

In conclusion, microbiome studies of particular environmental conditions or locations are yielding new insights into the diversity of microbes and their associations with other taxa. Here, we report a rare case of a non-harmful mycobiome on the exoskeleton of a particular order of arthropods. Fungal growth seems to be

promoted or, at least, tolerated by the animal. Such associations between arthropods and fungi have rarely been investigated. Our results indicate that non-pathogenic, i.e. commensal or mutualistic, relationships between arthropods and fungi may be much more widespread than is currently known.

Conflicts of interest

The authors declare no competing financial interests.

Author contributions

JOW, MS, SLG, PSD and AI conceived and designed the study. MS collected, identified, bred and supplied the whip spiders. JOW and SNG performed the scanning electron microscopy survey. ATG, AI, PSD and MK isolated and characterised the fungal strains. JOW, ATG, AI and PSD wrote the paper and all other authors equally contributed in revision.

Funding

This study was supported by a Biotechnology and Biological Sciences Research Council (UK) DTP studentship to ATG and a Macquarie University Research Fellowship of Macquarie University to JOW.

Acknowledgements

We thank Frank Kempken (University of Kiel) and Martín Ramírez (Natural History Museum of Argentina) for discussions on this topic. We would also like to thank the editor and the anonymous reviewers for inputs into the potential roles of the fungi in the Amblypygid interaction. We thank Nicolle Vella from the Microscopy Unit, Faculty of Science and Engineering, Macquarie University, for help and technical assistance with microscopy. We thank Esther Appel (University of Kiel) for technical advice on the preparation of digested sectioned cuticle samples. Thanks to Alan Henderson (Minibeasts Wildlife) for his cooperation in collecting whip spiders with our special requirements. We thank Peter Jäger (Senckenberg Research Institute Frankfurt) and Jan Beccaloni (Natural History Museum London) for access to museum collections and providing material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.05.003.

References

Aanen, D.K., Eggleton, P., Rouland-Lefevre, C., Guldberg-Frøslev, T., Rosendahl, S., Boomsma, J.J., 2002. The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proc. Natl. Acad. Sci. 99, 14887–14892.

Abdel-Baky, N.F., 2000. *Cladosporium* spp. an entomopathogenic fungus for controlling whiteflies and aphids in Egypt. Pakistan J. Biol. Sci. 3, 1662–1667.

Affokpon, A., Coyne, D.L., Htay, C.C., Agbèdè, R.D., Lawouin, L., Coosemans, J., 2011. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. Soil Biol. Biochem. 43, 600–608.

Angelo, I.C., Fernandes, É.K., Bahiense, T.C., Perinotto, W.M., Moraes, A.P.R., Terra, A.L., Bittencourt, V.R.E.P., 2010. Efficiency of *Lecanicillium lecanii* to control the tick *Rhipicephalus microplus*. Vet. Parasitol. 172, 317–322.

Askary, H., Benhamou, N., Brodeur, J., 1999. Ultrastructural and cytochemical characterization of aphid invasion by the hyphomycete *Verticillium lecanii*. J. Invertebr. Pathol. 74, 1–13.

Baby, S., Ramya, T.G., Geetha, R.K., 2015. Onychomycosis by *Syncephalastrum racemosum*: case report from Kerala, India. Dermatol. Rep. 7, 5527.

Barth, F.G., 1973. Microfiber reinforcement of an arthropod cuticle. Laminated composite material in biology. Z. Zellforsch. Mik. Anat. 144, 409–434.

Batra, S.W., Batra, L.R., 1967. The fungus gardens of insects. Sci. Am. 217, 112–124.

Bawin, T., Seye, F., Boukraa, S., Zimmer, J.Y., Raharimalala, F.N., Zune, Q., Ndiaye, M., Delvigne, F., Francis, F., 2016. Production of two entomopathogenic *Aspergillus* species and insecticidal activity against the mosquito *Culex quinquefasciatus* compared to *Metarhizium anisopliae*. Biocontrol Sci. Technol. 26, 617–629.

Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W., 2012. The genus *Cladosporium*. Stud. Mycol. 72, 1–401.

Bensch, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., de Jesus Yanes-Morales, M., Crous, P.W., 2015. Common but different: the expanding realm of *Cladosporium*. Stud. Mycol. 82, 23–74.

Bensch, K., Groenewald, J.Z., Meijer, M., Dijksterhuis, J., Jurjević, Ž., Andersen, B., Houbraken, J., Crous, P.W., Samson, R.A., 2018. *Cladosporium* species in indoor environments. Stud. Mycol. 89, 177–301.

Berendsen, R.L., Baars, J.J., Kalkhove, S.I., Lugones, L.G., Wösten, H.A., Bakker, P.A., 2010. *Lecanicillium fungicola*: causal agent of dry bubble disease in white-button mushroom. Mol. Plant Pathol. 11, 585–595.

Bibbs, C., Vitoreli, A., Benny, G., Harmon, C., Baldwin, R., 2013. Susceptibility of *Latrodectus geometricus* (Araneae: Theridiidae) to a Mucor strain discovered in north central Florida, USA. Fla. Entomol. 96, 1052–1061.

Bloch, C.P., Weiss, L., 2002. Distribution and abundance of the whip spider *Phrynus longipes* (Arachnida: Amblypygi) in the Luquillo Experimental Forest, Puerto Rico: response to natural and anthropogenic disturbance. Caribb. J. Sci. 38, 260–262.

Blackwell, M., 2011. The Fungi: 1, 2, 3… 5.1 million species? Am. J. Bot. 98, 426–438.

Boomsma, J.J., Jensen, A.B., Meyling, N.V., Eilenberg, J., 2014. Evolutionary interaction networks of insect pathogenic fungi. Annu. Rev. Entomol. 59, 467–485.

Capinera, J.L. (Ed.), 2008. Encyclopedia of Entomology. Springer Science & Business Media.

Carbone, I., Kohn, L.M., 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–556.

Castrillo, L.A., Griggs, M.H., Ranger, C.M., Reding, M.E., Vandenberg, J.D., 2011. Virulence of commercial strains of *Beauveria bassiana* and *Metarhizium brunneum* (Ascomycota: Hypocreales) against adult *Xylosandrus germanus* (Coleoptera: Curculionidae) and impact on brood. Biol. Control 58, 121–126.

Chandler, D., Davidson, G., Pell, J.K., Ball, B.V., Shaw, K., Sunderland, K.D., 2000. Fungal biocontrol of Acari. Biocontrol Sci. Technol. 10, 357–384.

Chandler, D., Davidson, G., Jacobson, R.J., 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. Biocontrol Sci. Technol. 15, 37–54.

Chapin, K.J., 2014. Microhabitat and spatial complexity predict group size of the whip spider *Heterophrynus batesii* in Amazonian Ecuador. J. Trop. Ecol. 30, 173–177.

Chapin, K.J., Hebets, E.A., 2016. The behavioral ecology of amblypygids. J. Arachnol. 44, 1–14.

Chen, A.J., Hubka, V., Frisvad, J.C., Visagie, C.M., Houbraken, J., Meijer, M., Varga, J., Demirel, R., Jurjević, Ž., Kubátová, A., Sklenář, F., 2017. Polyphasic taxonomy of *Aspergillus* section *Aspergillus* (formerly Eurotium), and its occurrence in indoor environments and food. Stud. Mycol. 88, 37–135.

Claydon, N., Grove, J.F., 1982. Insecticidal secondary metabolic products from the entomogenous fungus *Verticillium lecanii*. J. Invertebr. Pathol. 40, 413–418.

de Faria, M.R., Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control 43, 237–256.

Eken, C., Hayat, R., 2009. Preliminary evaluation of *Cladosporium cladosporioides* (Fresen.) de Vries in laboratory conditions, as a potential candidate for biocontrol of *Tetranychus urticae* Koch. World J. Microbiol. Biotechnol. 25, 489.

Evans, H.C., 2013. Fungal pathogens of spiders. In: Nentwig, W. (Ed.), Spider Ecophysiology. Springer, pp. 107–121.

Fernandes, É.K.K., Bittencourt, V.R.E.P., 2008. Entomopathogenic fungi against South American tick species. Exp. Appl. Acarol. 46, 71–93.

Ferron, P., 1978. Biological control of insect pests by entomogenous fungi. Ann. Rev. Entomol. 23, 409–442.

Fisher, P.J., Stradling, D.J., Sutton, B.C., Petrini, L.E., 1996. Microfungi in the fungus gardens of the leaf-cutting ant *Atta cephalotes*: a preliminary study. Mycol. Res. 100, 541–546.

Flannigan, B., Samson, R.A., Miller, D.J., 2016. Microorganisms in Home and Indoor Work Environments: Diversity, Health Impacts, Investigation and Control. CRC Press.

Frisvad, J.C., Samson, R.A., 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud. Mycol. 49, 1–174.

Foelix, R.F., 1979. Biology of Spiders. Georg Thieme Verlag.

Gallou, A., Serna-Domínguez, M.G., Berlanga-Padilla, A.M., Ayala-Zermeño, M.A., Mellín-Rosas, M.A., Montesinos-Matías, R., Arredondo-Bernal, H.C., 2016. Species clarification of *Isaria* isolates used as biocontrol agents against *Diaphorina citri* (Hemiptera: Liviidae) in Mexico. Fungal Biol. 120, 414–423.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118.

Gerson, U., Gafni, A., Paz, Z., Sztejnberg, A., 2008. A tale of three acaropathogenic fungi in Israel: *Hirsutella*, *Meira* and *Acaromyces*. Exp. Appl. Acarol. 46, 183–194.

Gindin, G., Barash, I., Harari, N., Raccah, B., 1994. Effect of endotoxic compounds isolated from *Verticillium lecanii* on the sweetpotato whitefly, *Bemisia tabaci*. Phytoparasitica 22, 189–196.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323–1330.
Goettel, M.S., Koike, M., Kim, J.J., Aiuchi, D., Shinya, R., Brodeur, J., 2008. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J. Invertebr. Pathol. 98, 256–261.
Gonçalves-Souza, T., Giupponi, A.P., Hernandes, F.A., 2014. A rarefinding of mites (Arachnida: Acari: Leeuwenhoekiidae) parasitizing a whip spider (Arachnida: Amblypygi: Charinidae). Folia Parasitol. 61, 182–184.
Graham, K., 1967. Fungal-insect mutualism in trees and timber. Annu. Rev. Entomol. 12, 105–126.
Greenstone, M.H., Ignoffo, C.M., Samson, R.A., 1987. Susceptibility of spider species to the fungus *Nomuraea atypicola*. J. Arachnol. 15, 266–268.
Hajek, A.E., 1997. Ecology of terrestrial fungal entomopathogens. In: Advances in Microbial Ecology. Springer, Boston, MA, pp. 193–249.
Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43.
Harms, D., 2018. A new species of Charinus (Amblypygi: Charinidae) from Ghana, with notes on West African whip spiders. Evol. Syst. 2, 45.
Huang, W.K., Sun, J.H., Cui, J.K., Wang, G.F., Kong, L.A., Peng, H., Chen, S.L., Peng, D.L., 2014. Efficacy evaluation of fungus *Syncephalastrum racemosum* and nematicide avermectin against the root-knot nematode *Meloidogyne incognita* on cucumber. PLoS One 9, e89717.
Hughes, D.P., Andersen, S.B., Hywel-Jones, N.L., Himaman, W., Billen, J., Boomsma, J.J., 2011. Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. BMC Ecol. 11, 13.
Irigaray, F.J.S.D.C., Marco-Mancebón, V., Pérez-Moreno, I., 2003. The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the twospotted spider mite *Tetranychus urticae*. Biol. Control 26, 168–173.
Jackson, M.A., Dunlap, C.A., Jaronski, S.T., 2010. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. BioControl 55, 129–145.
Jassim, H.K., Foster, H.A., Fairhurst, C.P., 1990. Biological control of Dutch elm disease: larvicidal activity of *Trichoderma harzianum, T. polysporum* and *Scytalidium lignicola* in *Scolytus scolytus* and *S. multistriatus* reared in artificial culture. Ann. Appl. Biol. 117, 187–196.
Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery. Nat. Rev. Microbiol. 17, 167–180.
Kershaw, M.J., Moorhouse, E.R., Bateman, R., Reynolds, S.E., Charnley, A.K., 1999. The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insect. J. Invertebr. Pathol. 74, 213–223.
Koike, M., Higashio, T., Komori, A., Akiyama, K., Kishimoto, N., Masuda, E., Sasaki, M., Yoshida, S., Tani, M., Kuramoti, K., Sugimoto, M., 2004. *Verticillium lecanii* (*Lecanicillium* spp.) as epiphyte and its application to biological control of arthropod pests and diseases. IOBC/wprs Bull. 27, 41–44.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Kramer, K.J., Muthukrishnan, S., 1997. Insect chitinases: molecular biology and potential use as biopesticides. Insect Biochem. Mol. Biol. 27, 887–900.
Lacey, L.A., Fransen, J.J., Carruthers, R., 1996. Global distribution of a naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. *Bemisia*: 1995. In: Taxonomy, Biology, Damage, Control and Management, pp. 401–433.
Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control 21, 230–248.
LeClerc, M.G., McClain, D.C., Black, H.L., Jorgensen, C.D., 1987. An inquiline relationship between the tailless whip-scorpion *Phrynus gervaisii* and the giant tropical ant *Paraponera clavata*. J. Arachnol. 15, 129–130.
Leles, R.N., Sousa, N.A., Rocha, L.F.N., Santos, A.H., Silva, H.H.G., Luz, C., 2010. Pathogenicity of some hypocrealean fungi to adult *Aedes aegypti* (Diptera: Culicidae). Parasitol. Res. 107, 1271–1274.
Luangsa-ard, J.J., Mongkolsamrit, S., Thanakitpipattana, D., Khonsanit, A., Tasanathai, K., Noisripoom, W., Humber, R.A., 2017. Clavicipitaceous entomopathogens: new species in *Metarhizium* and a new genus *Nigelia*. Mycol. Prog. 16, 369–391.
Ma, R., Chen, Q., Fan, Y.L., Wang, Q., Chen, S., Liu, X., Cai, L., Yao, B., 2017. Six new soil-inhabiting *Cladosporium* species from plateaus in China. Mycologia 109, 244–260.
Maxwell, M., 1978. Two rapid and simple methods used for the removal of resins from 1.0 μm thick epoxy sections. J. Microsc. 112, 253–255.
Mensah, R.K., Young, A., 2017. Microbial control of cotton pests: use of the naturally occurring entomopathogenic fungus *Aspergillus* sp.(BC 639) in the management of *Bemisia tabaci* (Genn.)(Hemiptera: Aleyrodidae) and beneficial insects on transgenic cotton crops. Biocontrol Sci. Technol. 27, 844–866.
Meyling, N.V., Eilenberg, J., 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol. Contr. 43, 145–155.
Murtagh, G., Dyer, P., McClure, P., Crittenden, P., 1999. Use of randomly amplified polymorphic DNA markers as a tool to study variation in lichen-forming fungi. Lichenologist 31, 257–267.
Nentwig, W., 1985. Parasitic fungi as a mortality factor of spiders. J. Arachnol. 13, 272–274.
Ownley, B.H., Gwinn, K.D., Vega, F.E., 2010. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl 55, 113–128.
Paoletti, M., Rydholm, C., Schwier, E.U., Anderson, M.J., Szakacs, G., Lutzoni, F., Debeaupuis, J.-P., Latgé, J.-P., Denning, D.W., Dyer, P.S., 2005. Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Curr. Biol. 15, 1242–1248.
Pitkin, J.W., Panaccione, D.G., Walton, J.D., 1996. A putative cyclic peptide efflux pump encoded by the *TOXA* gene of the plant-pathogenic fungus *Cochliobolus carbonum*. Microbiology 142, 1557–1565.
Porto, T.J., Peixoto, P.E.C., 2013. Experimental evidence of habitat selection and territoriality in the Amazonian whip spider *Heterophrynus longicornis* (Arachnida, Amblypygi). J. Ethol. 31, 299–304.
Ramírez, M.J., 2014. The morphology and phylogeny of dionychan spiders (Araneae: Araneomorphae). Bull. Am. Mus. Nat. Hist. 390, 1–374.
Rayor, L.S., Taylor, L.A., 2006. Social behavior in amblypygids, and a reassessment of arachnid social patterns. J. Arachnol. 34, 399–421.
Roberts, D.W., 1966. Toxins from the entomogenous fungus *Metarhizium anisopliae* II. Symptoms and detection in moribund hosts. J. Invertebr. Pathol. 8, 222–227.
Roberts, D.W., Hajek, A.E., 1992. Entomopathogenic fungi as bioinsecticides. In: Frontiers in Industrial Mycology. Springer, Boston, MA, pp. 144–159.
Roberts, D.W., St Leger, R.J., 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Adv. Appl. Microbiol. 54, 1–70.
Samson, R.A., Evans, H.C., Latgé, J.-P., 2013. Atlas of Entomopathogenic Fungi. Springer Science & Business Media.
Sandoval-Denis, M., Gené, J., Sutton, D.A., Wiederhold, N.P., Cano-Lira, J.F., Guarro, J., 2016. New species of *Cladosporium* associated with human and animal infections. Persoonia 36, 281–298.
Santamarina, M.P., Roselló, J., Llacer, R., Sanchis, V., 2002. Antagonistic activity of *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom and *Trichoderma harzianum* Rifai isolates against fungi, bacteria and insects *in vitro*. Revista Iberoamericana de Micología 19, 99–103.
Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal Barcoding Consortium, 2012. Nuclear ribosomal internal transcribed spacer (ITS) regions as universal DNA barcode marker for fungi. Proc. Natl. Acad. Sci. U.S.A. 109, 6241–6246.
Shakeri, J., Foster, H.A., 2007. Proteolytic activity and antibiotic production by *Trichoderma harzianum* in relation to pathogenicity to insects. Enzym. Microb. Technol. 40, 961–968.
Shang, Y., Feng, P., Wang, C., 2015. Fungi that infect insects: altering host behavior and beyond. PLoS Pathog. 11, e1005037.
Shin, T.Y., Bae, S.M., Kim, D.J., Yun, H.G., Woo, S.D., 2017. Evaluation of virulence, tolerance to environmental factors and antimicrobial activities of entomopathogenic fungi against two-spotted spider mite, *Tetranychus urticae*. Mycoscience 58, 204–212.
Spatafora, J.W., Chang, Y., Benny, G.L., Lazarus, K., Smith, M.E., Berbee, M.L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T.Y., 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108 (5), 1028–1046.
St Leger, R.J., Staples, R.C., Roberts, D.W., 1993. Entomopathogenic isolates of *Metarhizium anisopliae, Beauveria bassiana*, and *Aspergillus flavus* produce multiple extracellular chitinase isozymes. J. Invertebr. Pathol. 61, 81–84.
Suarez, B., Rey, M., Castillo, P., Monte, E., Llobell, A., 2004. Isolation and characterization of PRA1, a trypsin-like protease from the biocontrol agent *Trichoderma harzianum* CECT 2413 displaying nematicidal activity. Appl. Microbiol. Biotechnol. 65, 46–55.
Suh, S.-O., McHugh, J.V., Pollock, D.D., Blackwell, M., 2005. The beetle gut: a hyperdiverse source of novel yeasts. Mycol. Res. 109, 261–265.
Tsai, C.C., Tzeng, D.S., Hsieh, S.P.Y., 2008. Biological control of Fusarium stem rot of *Anoectochilus formosanus* Hayata by *Trichoderma asperellum* TA strain. Plant Pathol. Bull. 17, 243–254.
Vanderwolf, K.J., Malloch, D., McAlpine, D.F., 2016. Ectomycota associated with arthropods from bat hibernacula in Eastern Canada, with particular reference to *Pseudogymnoasucs destructans*. Insects 7, 16.
Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F., Rehner, S.A., 2008. Entomopathogenic fungal endophytes. Biol. Contr. 46, 72–82.
Vega, F.E., Meyling, N.V., Luangsa-ard, J.J., Blackwell, M., 2012. Fungal entomopathogens. Insect Pathol. 2, 171–220.
Viquez, C., de Armas, L.F., 2009. Parasitismo en huevos de amblipígidos (Arachnidi: Amblypygi) por moscas Chloropidae (Insecta: Diptera). Boletín de la Sociedad Entomológica Aragonesa 45, 541–542.
Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.-B., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A., 2014. Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78, 343–371.
Weber, N.A., 1966. Fungus-growing ants. Science 153, 587–604.
Weygoldt, P., 2000. Whip Spiders (Chelicerata: Amblypygi): Their Biology, Morphology, and Systematics. Apollo Books, Stenstrup, Denmark.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc. Guide Methods Appl. 18, 315–322.

Wolff, J.O., Seiter, M., Gorb, S.N., 2015a. Functional anatomy of the pretarsus in whip spiders (Arachnida, Amblypygi). Arthropod Struct. Dev. 44, 524–540.
Wolff, J.O., Huber, S.J., Gorb, S.N., 2015b. How to stay on mummy's back: morphological and functional changes of the pretarsus in arachnid postembryonic stages. Arthropod Struct. Dev. 44, 301–312.
Wolff, J.O., Gorb, S.N., 2016. Attachment Structures and Adhesive Secretions in Arachnids. Springer International Publishing, Cham, Switzerland.
Wolff, J.O., Schwaha, T., Seiter, M., Gorb, S.N., 2016. Whip spiders (Amblypygi) become water-repellent by a colloidal secretion that self-assembles into hierarchical microstructures. Zool. Lett. 2, 23.
Wolff, J.O., Seiter, M., Gorb, S.N., 2017. The water-repellent cerotegument of whip-spiders (Arachnida: Amblypygi). Arthropod Struct. Dev. 46, 116–129.
Yang, Y., Zhang, Y., Wang, M., Li, S.S., Ma, X.Y., Xu, Z.H., 2015. Bioefficacy of entomopathogenic *Aspergillus* strains against the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae). Appl. Entomol. Zool. 50, 443–449.
Yoder, J.A., Benoit, J.B., Christensen, B.S., Croxall, T.J., Hobbs, H., 2009. Entomopathogenic fungi carried by the cave orb weaver spider, *Meta ovalis* (Araneae, Tetragnathidae), with implications for mycoflora transfer to cave crickets. J. Cave Karst Stud. 71, 116–120.
Yoshioka, Y., Ichikawa, H., Naznin, H.A., Kogure, A., Hyakumachi, M., 2012. Systemic resistance induced in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1, a microbial pesticide of seedborne diseases of rice. Pest Manag. Sci. 68, 60–66.